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SCIENTIFIC MEMOIRS

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OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

MEDITERRANEAN FEVER IN INDIA: ISOLATION OF THE MICROCOCCUS MELITENSIS.

CAPTAIN GEORGE LAMB, M.D., I.M.S.

ASSISTANT SURGEON M. KESAVA PAI, M.B., C.M. (Madras).

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA
BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT
OF INDIA, SIMLA.



H 111 S 41 2006

CALCUTTA:

OFFICE OF THE SUPERINTENDENT OF GOVERNMENT PRINTING, INDIA.

1906.



Henry der Dodge memorial.

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1906.

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MEDITERRANEAN FEVER IN INDIA: ISOLATION OF THE MICROCOCCUS MELITENSIS*.

SINCE Wright's 'discovery in 1897 that the serum of patients who are suffering from or who have recovered from Mediterranean fever has a specific agglutinating reaction on M. Melitensis this method of diagnosis has been applied to the elucidation of the continued Indian fevers. Thus Wright and Smith have tabulated to cases invalided from India, in which the serum gave a sedimentation reaction with M. Melitensis in dilutions ranging in most cases from 1 in 150 to 1 in 300. Many of these cases at the time of the examination presented the usual sequelæ of the disease. At a later date 10 other examples were observed by Birt and Lamb in the Royal Victoria Hospital, Netley, in patients who had been invalided for such diseases as malaria, typhoid fever and rheumatism. It is worthy of remark that 14 out of the total of 20 cases thus observed came from Mian Mir; the other places where infection had occurred were Calcutta, Sabathu and Nowshera.

In 1900 one of us working in Bombay had the opportunity of studying five cases of Mediterranean fever, which had evidently been contracted in that city. These were cases of irregular fever without definite symptoms, although marked sweating and pains in the joints were present in all instances. A definite diagnosis was arrived at only by means of the serum agglutination reaction.

A short time afterwards four cases were diagnosed in Simla.⁵ All these showed clear clinical symptoms and the sera of two of the patients gave positive reactions with M. Melitensis. The sera of the other two cases were not examined.

In 1901 Greig or reported three cases of Mediterranean fever occurring in the Swat valley. This diagnosis was reached by means of the serum agglutination reaction, although the clinical symptoms were also very suggestive.

In 1904 Konrich,7 working in Kolle's laboratory, made a series of agglutination experiments with M. Melitensis, which consisted in testing the agglutination and sedimentation reaction of seven strains of M. Melitensis with the sera

[•] This paper was received for publication on August 22nd, 1905.

of 17 men, many of whom had been treated with tuberculin. He records having in some instances observed sedimentation in a dilution of 1 in 500; in dilution of 1 in 10 and 1 in 20 nearly all gave complete sedimentation. It was also noted that all the strains used did not give the same results, but that no difference was observed between patients previously treated with tuberculin and those not so treated. On this point Konrich's results are in direct opposition to those obtained by Birt and Lamb, 8 who in a series of over 150 controls found no sedimentation in dilution above 1 in 10, and also to those of M. C. Nicolle, 9 who, after an examination of the sera of 35 persons suffering from other diseases, concluded that a complete reaction in dilution of 1 in 10 was strong evidence of the case being one of Mediterranean fever.

Cornwall "examined the blood of nine healthy men and of nine cases of long-continued, but ill-defined, fever which were clinically not characteristic of Mediterranean fever, as regards their agglutination reaction with M. Melitensis. He relates having got in some of these cases a complete or partial reaction in dilution varying from 1 in 20 to 1 in 400, and concludes by saying "that even in dilutions of 1—80 a complete agglutination of M. Melitensis is not pathognomonic of Malta fever and that the blood of persons suffering from various febrile conditions may have a higher agglutination power on the micrococcus than that of persons in good health."

In spite, however, of the observations of Konrich and Cornwall we still hold, that the serum agglutination method for the diagnosis of Mediterranean fever, when properly and rigidly controlled, is one of the most delicate bacteriological tests with which we are acquainted and ought to take its place as an important addition to the ordinary clinical methods for the diagnosis of this disease. This opinion is evidently shared by the Commission "for the investigation of Mediterranean fever which is at present at work under the supervision of an Advisory Committee of the Royal Society. A perusal of the first two parts of their report, the only parts which have as yet appeared, will show the great use which these workers make of this test both in the diagnosis of cases and as a means of identifying the specific coccus. This opinion is strengthened by the result of the investigations which we have now to bring forward.

These investigations are concerned with observations made on several cases of continued fever in India, in which the diagnosis of Mediterranean fever was arrived at by means of the serum sedimentation test. Further, in a considerable number of these cases this diagnosis was proved to be correct by the isolation from the spleen during life of a coccus bacteriologically indistinguishable from M. Melitensis. As the preliminary step in all our cases consisted in testing the serum as regards its agglutinin content, and as much reliance was placed on the serum reactions of the specific organism when isolated from the spleen, it will be convenient

here, once and for all, to mention the technique employed and to state the precautions taken with a view to control these observations. All agglutination experiments were made macroscopically in the capillary tubes devised by Wright, by means of which also the serum dilutions were effected. Equal quantities of the various dilutions of serum, made with normal salt solution, and of sterile emulsions of the micrococcus were employed in all instances. The preparations were allowed to stand at laboratory temperature (about 18° C.) for 24 hours, when the results were recorded. The emulsions were all made in exactly the same way, namely, an uniform growth on agar of from 4 to 7 days was emulsified with sterile normal salt solution, about 2.5 cc. of the latter being used for each agar culture; the bacteria were then killed by heating at 60° C. for 15 minutes and finally 0.5 per cent. carbolic acid was added.

During the period that the observations to be related anon were in progress controls with the bloods of healthy individuals and of persons suffering from other diseases were made almost daily. In this way an ample number of control experiments were obtained both with the stock organism and with the several races isolated from our cases. Thus, we tested as controls the following bloods:—

(1) The bloods of 51 healthy individuals, all natives of India.—Of these, 11 were servants at the Pasteur Institute and 40 were persons who were undergoing inoculation against rabies. In the case of one of the latter individuals a complete reaction in dilution of 1 in 160 was obtained both with the stock culture and with one of the races isolated by us. An enquiry into the history of this person elicited the information that one and a half months previous to our examination of his blood he had suffered from remittent fever for at least a fortnight. During this illness he was at home in histvillage and did not come under medical observation. But he states that there were no objective symptoms except fever and profuse sweating.

In the remaining 50 cases no reaction was obtained in dilution of 1 in 10 with the stock M. Melitensis or with any of the strains isolated in India.

(2) All bloods sent to the Pasteur Institute of India for serum diagnosis of typhoid fever.—During the period over which our present investigation lasted more than 150 such bloods were examined with the emulsions of M. Melitensis which were in daily use. Not one of these bloods gave a trace of agglutination reaction in dilution of 1 in 20, the only dilution in which the preparations were put up.

These two series of control experiments, amounting in all to over 200 observations with different races of M. Melitensis, support our contention that this bacteriological test is a delicate one and that it affords an easy and absolutely trustworthy method of diagnosis between this disease and other fevers which clinically simulate it.

The cases of Mediterranean fever which have come under our investigation at the present time naturally fall into two groups:

- (a) those in which M. Melitensis was isolated from the spleen during life; and,
- (b) those in which no splenic puncture was made, the diagnosis resting solely on the clinical history and the serum agglutination reaction.

Group a.—The cases in this class number eleven. In these cases a few drops of blood removed from the spleen during life with a sterile syringe were spread over several agar tubes. This simple operation was done under our written instructions by the medical officer in charge of the case. The agar tubes thus inseminated were at once returned to the laboratory, where the further examination of the growths was made. In nearly every instance a pure culture of a coccus, presently to be described, was obtained. In one or two cases only were there any contaminating organisms and these were evidently skin cocci of different kinds. No difficulty, however, was ever met with in isolating the specific organism from these tubes.

From all the cases which gave a serum agglutination reaction with M. Melitensis and in which splenic puncture was made there was isolated from the spleen, by the method described, a small coccus which presented the following characteristics, which are regarded by the workers on the Commission for the investigation of Mediterranean fever as justifying the organism being with confidence regarded as M. Melitensis:—

- (1) A slow growth at 37° C. on agar slopes; discrete colonies are circular, transparent and dew-like; the complete surface-growth is greyishwhite and moist; when cultures are old the growth often acquires a yellowish-brown colour.
- (2) Stained by weak carbol-fuchsine shows a small coccus or cocco-bacillus, appearing mostly as diplo-cocci and in short chains.
- (3) Stained by Gram-Weigert method does not retain its stain.
- (4) Grown in litmus milk, there is no development of gas, acidity or coagulation, but there is a production of alkalinity.
- (5) Grown on glucose-litmus agar there is no production of acidity, but production of alkalinity.
- (6) Agglutinated and sedimented with the serum of an animal immunised with a pure standard growth of M. Melitensis.
- (7) In the case of two of the races isolated a typical infection of a monkey was produced.

To points 6 and 7 we shall return later. Having, therefore, stated that the various strains of M. Melitensis isolated by us responded to the above tests, we may now consider the cases from which this organism was isolated more or less in detail.

Case I.—Sher Hussain; aged 24, Sepoy, 27th Punjabis, Multan. Patient was admitted into hospital on the 4th March 1905. From this date fever of a remittent type (vide chart I) continued for six weeks, when he died of exhaustion.

There were no marked symptoms except constipation and profuse sweating. No joint pains were complained of. Quinine even in large doses was without any influence.

The agglutination reaction of the serum with M. Melitensis was tested on two occasions, namely, at the beginning of the third week and in the middle of the sixth week. On the first occasion a complete reaction with stock M. Melitensis was got in dilution of 1 in 1200. On the second occasion the reaction was tested both with the stock strain and with the race isolated from the patient's own spleen. This latter organism will hereafter be known as Multan I. The following was the result of this examination:—

	Di	lution (of serui	m.			Stock.		Multan I.		
1—400		,						imenta-		imenta-	
1—800				*		•	Complete	"	Complete	2)	
1—1600							Well marked	,,	Complete	33	
1-3200			+		*		Trace of	33	Well marked	**	
1-6400							No	"	No	"	

Blood was taken from the spleen on two separate occasions, namely, in the middle of the fifth week and at the end of the sixth week. On both occasions a pure culture of M. Melitensis was obtained (Multan I). This organism was used for the inoculation of a monkey to be referred to hereafter.

Case II.—Hakim Singh; aged 22, Sepoy, 27th Punjabis, Multan. Patient was admitted into hospital on 11th March 1905. He was at first treated as a case of malaria, but the fever failed to yield to the administration of large doses of quinine. From the chart (vide chart II) it is seen that the temperature was very erratic throughout. It came to normal about the end of the sixth week, the man being sent on furlough soon afterwards. During the third week patient complained of acute pain in the left hip-joint. There were no other characteristic symptoms.

The serum reaction with M. Melitensis was tested on two occasions, namely, about the end of the fourth week and again one week later. On the first

occasion it gave a complete reaction with the stock M. Melitensis in dilution 1 in 640. On the second occasion the following was the result:—

	D	ilution	of seru	m.		Stock.	Multan I.		
1—40	٠	٠			•	Complete sedimenta-	Complete sedimenta-		
1—80						Complete "	Complete "		
1-200	•					Well marked "	Complete "		
1-400					٠	Trace of ,,	Well marked "		
1-800						No "	Trace of "		

Blood was withdrawn from the spleen about the end of the fifth week. A pure culture of M. Melitensis was obtained (Multan II).

Case III.—Teja Singh; aged 36, Sepoy, 27th Punjabis, Multan. Patient was admitted into hospital on the 24th March 1905 suffering from high fever. The temperature (vide chart III) fell gradually and reached normal on the 12th day. Patient was then discharged to duty. After an apyretic interval of about a fortnight's duration, a relapse, which lasted for several weeks, began. The temperature during this relapse was markedly remittent. No other noteworthy symptoms were present.

The agglutination reaction of the serum with M. Melitensis was tested on two occasions. On the first occasion, five days after the onset of the initial attack, a complete reaction in dilution of 1 in 60 was obtained with an emulsion of stock M. Melitensis. On the second occasion, namely, at the end of the fourth week and shortly after the beginning of the relapse, the following result was got:—

	D	ilution	of seru	ım.		Stock.	M	Multan.		
1—40						Complete sedimenta	Complete	sedimenta-		
1—8o						Well marked "	Complete			
1-160						No "	No	"		

Blood was taken from the spleen at the beginning of the fifth week. A pure growth of M. Melitensis was got on all the tubes inseminated (Multan III).

Case IV.—Sahib Singh; aged 29, Sepoy, 27th Punjabis, Multan. Patient was admitted into hospital on 31st March 1905 suffering from fever. The temperature (vide chart IV) was of a markedly remittent type. After nearly nine

weeks' illness patient was sent on sick leave, the temperature not yet having come to normal. There were no other prominent symptoms.

The serum was examined as regards its agglutination reaction with M. Melitensis at the beginning of the third week. The following was the result:—

	Di	lution o	of serui	m.		St	ock.	Multan I.		
1—40						Complete	sedimenta-	Complete se	dimenta-	
1—80						Complete	"	Complete	"	
1—120			4			Complete	"	Complete	,,	
1-240						Trace of	"	Well marked	,,	
1-480						No	"	Trace of	"	
1—96o						No	"	No	,,	

Blood was with drawn from the spleen at the beginning of the fourth week. Agar tubes sown with this blood gave a pure growth of M. Melitensis (Multan IV).

Case V.—Secunder Khan; aged 27, Sepoy, 27th Punjabis, Multan. Patient was admitted into hospital on 20th March 1905 suffering from fever and bronchitis. The bronchitis soon passed off, but the fever, which was markedly intermittent (vide chart V) continued. The temperature came to normal about the beginning of the fourth week and the patient was shortly afterwards sent on sick leave.

The agglutination reaction of the serum with M. Melitensis, tested at the end of the third week, was as follows:—

	D	ilution	of seru	m.			Stock.		Multan I.		
ı—8o					,		Complete sed	imenta-	Complete	sedimenta-	
1—160							0 1	"	Complete	,,	
1—320							Complete	,,	Complete	,,,	
1-640							Complete	"	Complete	23	
1—1280							Well marked	"	Complete	,,,	
1-2560						7.	Trace of	2)	Trace of	,,	
1-5120							No	,,	No		

Blood was taken from the spleen on the second day of the fourth week, namely, the first day on which the temperature was normal. A pure culture of M. Melitensis was obtained (Multan V).

Case VI.—Bishan Singh (1); aged 20, Sepoy, 27th Punjabis, Multan. Patient was admitted into hospital on 30th March 1905 suffering from fever of an intermittent type. During the second week the temperature was markedly remittent, but came to normal about the end of this week. There was now an apyretic interval of exactly seven days. This was followed by a severe relapse of over three weeks' duration. The temperature ultimately came to normal about the beginning of the seventh week.

The agglutination reaction of the serum was tested with M. Melitensis at the end of the third week, that is to say, at the very beginning of the relapse.

The	fol	lowing	was	the	resu	lt	-
T 110	101	SITT AL OF	MA CEN	CIIC	. 000	-	

	D	ilution	of seru	m.		St	ock.	Multan I.		
1—40					4		sedimenta-	Complete se	dimenta	
1—80						Complete	,,	Complete	"	
1—160						Complete		Complete	***	
1-320						Trace of	"	Well marked	"	
1-640	4	4				No	- 29	No	2)	

Blood was taken from the spleen at the beginning of the sixth week. M. Melitensis was isolated in pure culture from the tubes inseminated (Multan VI).

Case VII.—Bishan Singh (2); aged 34, Sepoy, 27th Punjabis, Multan. Patient was in hospital in Ferozepore under treatment for "remittent fever" from 5th September till 2nd November 1904. He was then sent back to duty. No chart or detailed record of this illness is available. On the 1st May 1905, six months after his discharge, he was again admitted into hospital suffering from fever. The temperature (vide chart VII) came to normal in seven days, and five days afterwards he was discharged to duty. For fourteen days after his discharge from hospital patient was under observation-and doing ordinary duty. He had no relapse during this period. He was then sent on furlough.

The agglutination reaction of the serum with M. Melitensis was tested on two occasions: first, on the fourth day after his admission to hospital and again

eight days after the temperature had come to normal. The result, the same on both occasions, was as follows:—

	D	ilution	of seru	m.		Stock-	Multan I.	
1—40						Complete sedimenta-	Complete sedimenta-	
1-80					11	Complete "	Complete "	
1-160						Well marked "	Well marked "	
1-320						No "	No "	

Blood was taken from the spleen eight days after the temperature fell to normal. In one tube a pure culture of M. Melitensis was obtained (Multan VII).

Case VIII.—Labh Singh; aged 23, Sepoy, 27th Punjabis, Multan. Patient was admitted into hospital on the 5th May 1905 suffering from fever which was of a remittent type (vide chart VIII) and persisted for seven weeks in spite of the exhibition of large doses of quinine. He complained at one time of slight pains in the knee-joints. Just before the temperature came to normal, patient suffered from such severe pain in the right hip-joint that he was almost unable to move. This pain lasted on and off for a month.

The serum agglutination reaction with M. Melitensis was tested on two occasions.

In the middle of the third week the result was as follows:-

	D	ilution	of seri	ım.		Stock.	Multan I.
1—40						Complete sediment	ca- Complete sedimenta-
1-80						 Well marked "	Well marked "
1-160					٠.	No "	Trace of "
1-320						No "	No "

Again tested at the beginning of the sixth week the following result was obtained:—

	Di	lution o	f seru	m.		St	ock.	- Multan I.		
1-40						Complete	sedimenta-	Complete	sedimenta-	
1-80						Complete	1)	Complete	,,	
1-160				15		Trace of	1)	Trace of	"	
1-320						No	,,	No	n	

Blood was withdrawn from the spleen at the beginning of the sixth week. The tubes were all contaminated with a white staplylococcus, but M. Melitensis was easily isolated. (Multan VIII).

Case IX.—Gulab Khan; aged 19, Sepoy, 27th Punjabis, Multan. Patient was admitted into hospital on 28th May 1905 suffering from continued fever which resisted large doses of quinine, (chart IX.) There was slight delirium at first, but this soon passed off. During the fifth week patient complained of severe pain in the right hip-joint: this pain lasted only for a few days. There were no other marked symptoms.

The serum agglutination reaction with M. Melitensis was tested about the end of the second week. The following was the result:—

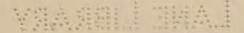
	D	ilution	of seru	m.		St	ock.	Multan I.		
1-40						Complete	sedimenta-	Complete	sedimenta-	
1—80						Complete	"	Complete	"	
1—160						Complete	"	Complete	,,	
1-320						Trace of	n	Trace of	,,	
1-640					4.	No	,, .	No	,,	

At the end of the second week blood was removed from the spleen and sown in agar. A pure culture of M. Melitensis was obtained (Multan IX).

Case X.—Dalhara Singh; aged 22, Sepoy, 15th, Sikhs, Ferozepore. Patient was admitted into hospital on 26th April 1905 suffering from fever of a remittent type. For temperature vide chart X. There were no other marked symptoms.

The serum agglutination reaction with M. Melitensis was estimated on two occasions. First, at the end of the second week. The following result was obtained:—

	Di	lution	of seru	m.		Stock.		Multan I.		
1—80						Complete sedir	menta-	Complete	sedimenta-	
1—160						C 1-4-)1	Complete	1)	
1-320						Complete	,,	Complete	"	
1-640						Well marked	,,	Complete	,,	
1-1280						No	,,	Trace of	"	
12560				100		No	,,	No	,,	



Again, at the end of the sixth week and a fortnight after the temperature had come to normal the serum tested with the same two emulsions gave the following reaction:—

	Dil	ution	of serui	n.		St	ock.	Multan I.	
1—80			4			Complete	sedimenta-	Complete	sedimenta-
1—160						Complete	"	Complete	"
1-320						Trace of	,,	Well marke	ed "
1-640		,				No	,,	Trace of	,,
1-1,280						No	"	No	,,

Blood was withdrawn from the spleen at the end of the second week. M. Melitensis was isolated in pure culture (Ferozepore I).

Case XI.—Mr. M.; aged 32, Ferozepore. Patient had been ten years resident in India before the present illness. He came under observation for the first time on 18th May 1905. Before this date he had suffered from fever for a week. For temperature vide chart XI. During the short time of which there is no temperature record the patient was removed from Ferozepore to the Murree Hills. He states, however, that during this period his temperature was above normal. The fever continued for several weeks after his arrival in the hills and at the time of writing is still present. Constipation was trouble-some throughout. Further, he complained a good deal of joint pains, first in the left shoulder, next in the right shoulder and then more generally in several of the other joints. Latterly severe pain was complained of in the right hip and back. There was no orchitis.

The serum reaction with M. Melitensis, first examined towards the beginning of the illness, namely on the tenth day, was as follows:—

	D	ilution	of seru	m.		Stock.	Mui	ltan I.
1—80						Complete sedimenta-	Complete	sedimenta-
1—160		*				Complete ,,	Complete	"
1-320						Complete "	Complete	
1-640						Well marked "	Complete	"
1-1,280						No "	No	,,

The serum reaction was tested with the same emulsions twice afterwards, namely, in the middle of the fifth week and towards the end of the sixth week. The result, the same on both occasions, was as follows:—

	Dil	ution o	of serun	n.		Stock.	Multan I.		
1—80				•		Complete sedimenta-	Complete sedimenta		
1—1,60						Complete , ,,	Complete "		
1-320	•					Complete "	Complete ,,		
1-640						Complete ,,	Complete "		
1—1280			•	+		Well marked "	Complete "		
1-2560			7.		10.	Trace of "	Well marked "		

Blood was taken from the spleen towards the end of the sixth week. M. Melitensis was isolated in pure culture (Ferozepore II).

Before passing on to record those cases in which no splenic puncture was made and in which the diagnosis rested mainly on the serum agglutination reaction, we have to draw attention to two of the tests, to which the microbes isolated from the cases described above were subjected.

We have already pointed out in passing that one of the tests, to which all the strains confirmed, was the serum agglutination test. This test was carried out in the following manner:—

Two rabbits were treated with intra-peritoneal injections of sterile emulsions of M. Melitensis, one with the race known as stock and the other with the organism isolated from Sher Hussain (Multan I). The former strain was procured about two years ago from the Royal Army Medical College, London, and probably had its origin from a case at Netley Hospital. After several injections the sera of these rabbits were tested against all the races which had been isolated by us. The following tables show the results obtained:—

Table I with the serum of the rabbit immunised with stock M. Melitensis.

Table II with the serum of the rabbit immunised with M. Melitensis,

Multan 1.

				RA	CES O	ĖМ. М	ELITE	NSIS.						
Dilution of	S	· .			1	MULTAN	•				Feroze	Ferozepore.		
serum.	Stock.	i	ii	iii	iv	▼	vi •	v ii	viii	ix	i	ii		
200	•	•	•	•		•	•	•	•	•	•	•		
400	•	•	•	*	•	•	•	•	•	•	•	•		
боо	•	•	•	•	•	•	•		•	•	•	٠		
800	•	•	•	•	*	+	.•	+	•	•	•	t		
1000	•	•	• •	+	• .	+	•	+	+	+	+	t		
1500	† +	‡	+	ŧ	‡	‡	‡	:	:	‡	:	1		
2000	:	§	‡	:	‡	§	§	§	§	§	§	9		
2500	§	§	§	§	§	§	§	§	§	§	§			

TABLE II.

Rabbit II. Immunised with emulsions of M. Melitensis Multan I.

				RA	CES OF	M. ME	LITEN	SIS.						
Dilution of	Stock.				1	MULTAN	•				Feroz	Ferozepore		
serum.	200 •	i	ii	iii	iv	•	vi	vii	viii	ig	i	ü		
	•		•	•	*	•	•	•	•	•	•	•		
400	•	•	•	•	•	•	•	•	•	•	•	•		
600	+		•	•	•	•	•	*	•	*	•	•		
800	+	*	. •	•	•	+	*	‡	• 1	•	*	,		
1000	7	•	*	+		t	+	t	?		•	,		
1500	5	1	†	1	:	:	‡	‡	‡	‡	‡	:		
2000	§	§	+	§	Į.	§	§	§	§	§	§			
2500	5	§	1	§.	§	§	§	§	§	§	§			

NOTE.—In these tables—

- * Denotes complete sedimentation.
- † Denotes well marked but not complete sedimentation.
- ‡ Denotes slight or trace of sedimentation.
- § Denotes no sedimentation.

It will be seen from these tables that all the races isolated by us gave good agglutination reactions with both sera.

We have also mentioned that in two instances a monkey was infected with a strain of M. Melitensis isolated by us. The following are the details of these two experiments.

Monkey I.—Received subcutaneously one agar tube of M. Melitensis, Multan I.

For temperature see chart. During the third week the animal was very ill; it would eat nothing and became very thin; it lay at the bottom of its cage and at one time appeared moribund. However, it gradually recovered and was quite well at the end of the ninth week; then it was killed with chloroform.

The serum agglutination reaction with two races of M. Melitensis was determined on three occasions.

First, before inoculation no reaction even in dilution of 1-4 was got with either stock or Multan I.

Secondly, on the sixth day after infection a complete reaction in dilution of 1—10 and a nearly complete sedimentation in dilution of 1-20 with both these strains were observed.

Thirdly, in the middle of the third week the following result was obtained:-

	Di	lution	of seru	m.		Si	tock.	Multan I.		
1—40						Complete	sedimenta-	Complete	sedimenta-	
1—80						Complete	,, -	Complete	"	
1—160						Complete	" "	Complete	,,	
1-320						Trace of	"	Complete	,,	
1-640						No	"	Complete	,,	
1-1280						No "	,,	Trace of	,,	

In the middle of the fourth week 1c.c. of blood was withdrawn from a superficial vein and planted in broth and on agar. A pure culture of M. Melitensis, which answered to all the tests mentioned above, was obtained. The animal was killed by chloroform at the end of the ninth week. Cultures made from the heart's blood, the liver and the spleen all remained sterile.

Monkey II.—Received subcutaneously one agar tube of M. Melitensis, Multan II.

There was a well marked local reaction at the site of inoculation; this resolved without abscess formation. The animal was never at any time as ill as the first monkey had been; it never refused food and, in fact, appeared always to be in fair health. For temperature see chart.

The serum agglutination reaction with three races of M. Melitensis was estimated on three occasions.

First, before inoculation no reaction was obtained in dilution of 1 in 4 with stock, Multan I or Multan II.

Secondly, at the beginning of the third week complete sedimentation in dilution of 1 in 40 was observed with all three strains.

Thirdly, at the beginning of the fourth week the following result was obtained:—

Dile	ution o	serum		Stock.	Multan I.	Multan II.		
1—80				Complete sedi- mentation.	Complete sedi -	Complete sedi- mentation.		
1-160				Complete sedi- mentation.	Complete sedi- mentation.	Complete sedi- mentation.		
1-320				Complete sedi- mentation.	Complete sedi- mentation.	Complete sedi mentation.		
1-640				Trace of sedi- mentation.	mentation.	mentation.		
1—1280				No sedimentation	No sedimentation	Trace of sedi		

These estimations show a great increase in the agglutinin content of the blood in the course of one week.

At the beginning of the fifth week 2 c.c. of blood were removed from a peripheral vein and planted in broth and on agar. A pure growth of M. Melitensis was got from one of the broth tubes. The other tubes remained sterile.

At the end of the ninth week the animal was killed with chloroform. Cultures were made from the heart's blood, from the liver and from the spleen. The tubes inoculated with heart's blood remained sterile. A pure culture of M. Melitensis was obtained from both the liver and spleen. This organism responded to all the tests mentioned above.

Group b.—We have now briefly to refer to a few cases of continued fever, in which for various reasons no attempt was made to isolate the causal agent of the disease, but the sera of which gave a good agglutination reaction with M. Melitensis. These cases in the light of our recent researches we must regard as undoubted cases of Mediterranean fever.

Case XII.—Sohun, aged 32; Sepoy, 27th Punjabis, Multan. Patient was admitted into hospital on 6th March 1905 complaining of cough and fever.

The case at first was thought to be one of pneumonia, as there were slight physical signs at the base of both lungs. The lung condition, however, soon cleared up and at no time was it sufficient to account for the continued fever and the prostrate condition of the patient. Death took place from cardiac failure on the thirteenth day of illness.

On the eleventh day of the illness the serum agglutination reaction with M. Melitensis was tested by us. At that time the only strain available was the stock. With an emulsion of this race a complete reaction was obtained in dilution of 1 in 1200.

Case XIII.—Mian Jan; aged 23, Sepoy, 27th Punjabis, Multan. Patient was admitted into hospital on 17th April 1905 suffering from fever. The fever, which was of a remittent type (vide chart XIII) continued uninterruptedly for nearly seven weeks, when the patient was sent "away" or "placed on" on sick leave. There were no joint pains at any time. During the second week, when the evening temperature daily rose to about 103° F., there was considerable abdominal pain accompanied by vomiting. This, however, soon passed off under treatment.

The agglutination reaction of the serum with M. Melitensis was estimated on two occasions, first at the end of the second week, and again at the beginning of the fourth week. The result, the same on both occasions, was as follows:—

	D	ilution	of seru	m.		Stock. *	Multan I.	
1—80					1	Complete sedimenta-	tion.	
1-160						Complete "	Complete "	
1-320					0.0	Complete ,,	Complete ,,	
1-640						Well marked "	Complete ,,	
1-1280						Trace of "	Well marked "	

Case XIV.—Abdulla; aged 48, prisoner in Ferozepore Jail. Patient was admitted into hospital on 1st March 1905. On admission it was noted that there was slight fever accompanied with cough. The throat was somewhat congested. There was no enlargement of the spleen. The patient was under observation for more than ten weeks, when he left hospital on being discharged from Jail. During all this time the fever continued. (Vide chart XIV.) There were no marked symptoms. At times there were slight cough and expectoration, but no physical signs of disease could be detected in the lungs. The sputum was examined on several occasions for tubercle bacilli, but the

result of these examinations was always negative. No complaint of pain in the joints was made.

At the beginning of the tenth week the blood was sent to us for examination. The following was the result of the serum agglutination test with M. Melitensis:—

	I	Dilution	of serur	n.		Stock.		Multan I.		
1—40 1—80						C 1.	imenta- tion.	Complete Complete	sedimenta-	
1-160						Well marked	"	Complete	"	
1-320						No	,,	Trace of	,,	
1-640						No	,,	No	,,	

It was unfortunate that this patient was discharged from hospital before a splenic puncture could be done.

Case XV.—Mr. J. D.; aged 22, Lahore. Patient was admitted into hospital on the 5th May 1905 suffering from fever. For temperature vide chart XV. The fever, which was of a markedly remittent type, continued for more than ten weeks, when the patient passed out of observation. Neither the spleen nor liver was enlarged. Constipation was troublesome at intervals throughout the illness. During the fifth week the left wrist-joint became swollen and painful. No other joints were affected.

At the beginning of the ninth week the serum agglutination reaction with M. Melitensis was tested with the following result:—

Dilution of serum.								Multan I.			
1—80								114.1		Complete sedime	
1-160				4						Well marked ,,	
1-320		-								No-	

Case XVI.—Mrs. P., aged 28, Lahore. Patient was admitted into hospital on 26th May 1905 with a history of having suffered from fever for twelve days before admission. During the third week patient was very ill, the temperature on one occasion running up to over 105° F. (vide chart XVI). The spleen was slightly enlarged; the liver remained normal. Constipation was very troublesome throughout the illness, an enema as a rule being required to

procure an evacuation. Quinine was without effect. There were no joint pains complained of at any time.

The serum agglutination reaction with M. Melitensis was estimated in the Eddle of the seventh week. The following was the result:—

Dilution of serum.								Mul	tan I.		
1—8o				14						Complete	sedimenta-
1-160							14			Complete	"
1-320						18				No	111

Case XVII.—Amalak Shah, aged 17, an albino. Patient first came under medical observation on the 19th June 1905. He stated that he had been suffering from fever for a day or two before this date. He was resident in Murree. For temperature vide chart XVII. It will be seen that on occasions a high temperature, over 105° F., was recorded; its remittent nature was fairly well marked. Profuse perspiration was a prominent symptom throughout. About the middle of the fourth week he first complained of pain in the joints; at this time the right hip was affected and later on the joints of the toes became involved. Further, he complained of pain in the muscles of the thighs and back. Constipation and diarrhæa alternated. At one time faint sibilant rales were heard over both lungs. This bronchitis, however, soon passed off.

The serum reaction with M. Melitensis was tested in the middle of the third week with the following result:—

	Di	lution o	of seru	m.			Stock.	Multan 1.
1-80					90		Complete sedimenta-	Complete sedimenta
1—160						٠	Complete "	Complete "
1-320							Complete "	Complete "
r-640							Complete ,,	Complete "
1-1280							Well marked "	Complete "
1-2560							No "	Well marked "

This case completes the record of the cases of Mediterranean fever which have come under our observation at the present time. We have, however, still

to draw attention to one or two questions which have appeared during the course of the investigation.

In the first place, it is important to note the great differences in the agglutination value of the sera in the different cases. A complete reaction in some cases was obtained in no higher dilution than 1 in 40, while in other cases a complete reaction was observed in a dilution of 1 in 1200 or even higher. The significance of these variations has been already pointed out by Birt and Lamb 13 and by Bassett-Smith. 14

In the second place, it is of interest to note that M. Melitensis was isolated from the spleen at various stages of the disease. In most instances the temperature was still high and the illness in a more or less acute stage. But in case VI the temperature was falling and came to normal eight days afterwards; in case V the spleen was punctured on the first day of apyrexia; while in case VII the temperature, which had only been raised for seven days, had been subnormal for eight days before M. Melitensis was isolated from the spleen.

In the third place, case VII is of great interest. It will be remembered that this man was in hospital suffering from "remittent fever" from 5th September to 2nd November 1904. It is probable, in view of the facts which we shall refer to in a moment, that this illness was Mediterranean fever. For six months after this illness he was free from fever and able to do his duty as a sepoy in the regiment. After this interval he was again admitted into hospital suffering from fever, which only lasted for seven days. There were at this time no other symptoms. Now, the fact that M. Melitensis was isolated from the spleen eight days after the temperature came to normal is positive proof that this fever of short duration was an attack of Mediterranean fever. The question, then, is at once raised, as to whether this last illness was a relapse after six months' interval or a fresh infection. If it were a relapse, we have to assume that the causal bacterium had remained alive in the spleen or other internal organ for six months without giving rise to any symptom. If, on the other hand, it were a fresh infection, we have to conclude that one attack of Mediterranean fever does not confer immunity for any length of time. This latter conclusion is quite contrary to all clinical experience. We are, therefore, inclined to support the other view, namely, that we were here dealing with a relapse.

In the fourth place, there is an interesting point in the epidemiology of this disease which is raised by an analysis of our cases.

Of the 17 cases which we have recorded above, 11 occurred in one regiment, namely, the 27th Punjabis. We have seen that one of these cases was probably a relapse, the original attack having occurred some months previously. The remaining ten cases were admitted into hospital between the 4thMarch and 18th May 1905, but seven of these ten cases were admitted during the month

of March. At first sight it would appear easy to trace a common source of infection for these cases. But a careful investigation by the medical officer in charge of the regiment failed to bring forth any likely method of common infection. The men belonged to different companies, to different races in fact, slept in different barracks and practically never met.

When this small epidemic occurred the regiment was stationed at Multan in the Punjab. They arrived there from Ferozepore, also in the Punjab, on the 29th January 1905. On careful inquiry it was found out that shortly before leaving Ferozepore several cases of continued fever, some with a history of neuralgia and pain in the joints, had been treated in hospital. No chart or detailed records of these cases were available, but the bloods of a few of them were found to sediment M. Melitensis. These cases are as follows:—

- (1) Subadar Bhooroo.—He had been in hospital for 34 days suffering from fever and severe sciatica. His serum, tested six months afterwards, completely sedimented Multan I in dilution of 1 in 80.
- (2) Mancha Singh.—This man had been in hospital on three separate occasions suffering from fever with neuralgia and pains in the joints, namely:—

From (a) 8th June 1904 to 22nd June 1904;

- (b) 30th June 1904 to 26th August 1904;
- (c) 28th October 1904 to 5th December 1904.

The serum, tested five months after the last relapse, completely sedimented Multan I in dilution of 1 in 80.

(3) Surprag Khan.—This man was in hospital suffering from remittent fever from 21st July 1904 to 26th August 1904.

The serum, tested nine months afterwards, gave a complete reaction with Multan I in dilution of 1 in 40.

(4) Udho.—This man was in hospital suffering from fever, which was diagnosed as ague, from 25th July 1904 to 18th September 1904.

The serum, tested nine months afterwards, completely sedimented Multan I in dilution of 1 in 80.

(5) Lal Singh.—This man was in hospital from 22nd November 1904 to 5th January 1905 suffering from continued fever, which was also diagnosed as ague. The serum, tested four months afterwards, gave a complete reaction with Multan I in dilution of 1 in 80.

Further, the medical officer who was in charge of the regiment in Ferozepore was invalided to England after having suffered from a long continued remittent fever, which was regarded as being of malarial origin.

There can be no doubt, therefore, that the regiment while in Ferozepore suffered considerably from Mediterranean fever and that cases were occurring just before it left that station.

This regiment on its departure to Multan was succeeded in the same barracks in Ferozepore by the 15th Sikhs, which, however, did not arrive till the 4th April, more than two months after the 27th Punjabis had left. The 15th Sikhs came from the Samoena, on the Afghan frontier, where they had been stationed for a little over a year. During this time the records show that no cases at all resembling Mediterranean fever were treated in hospital, although while in Peshawar in 1903, before going to the Samoena, two cases, both clinically typical of the disease and in which the serum agglutination reaction with M. Melitensis was positive, had occurred. Such being the facts, it was, therefore, of the greatest interest to find a case of Mediterranean fever among the 15th Sikhs occurring about three weeks after the regiment had arrived in Ferozepore, namely, case X. We have made repeated enquiries from the medical officer of this regiment, and have examined the bloods of several cases of fever which have occurred recently, but this is the only case of Mediterranean fever which has been discovered.15 There is no doubt that this man became infected in Ferozepore. But the infection left behind by the 27th Punjabis cannot have been of a serious or widespread nature at the time of the arrival of the 15th Sikhs, otherwise we should have expected to find a more or less considerable epidemic breaking out among the new-comers. The probable explanation of this exemption is, we imagine, to be found in the fact that more than two months had elasped between the departure of the one regiment and the arrival of the other. This explanation receives support from the observation of Horrocks,16 that M. Melitensis survives for 43 days in soil allowed to dry naturally and for 72 days in a damp soil.

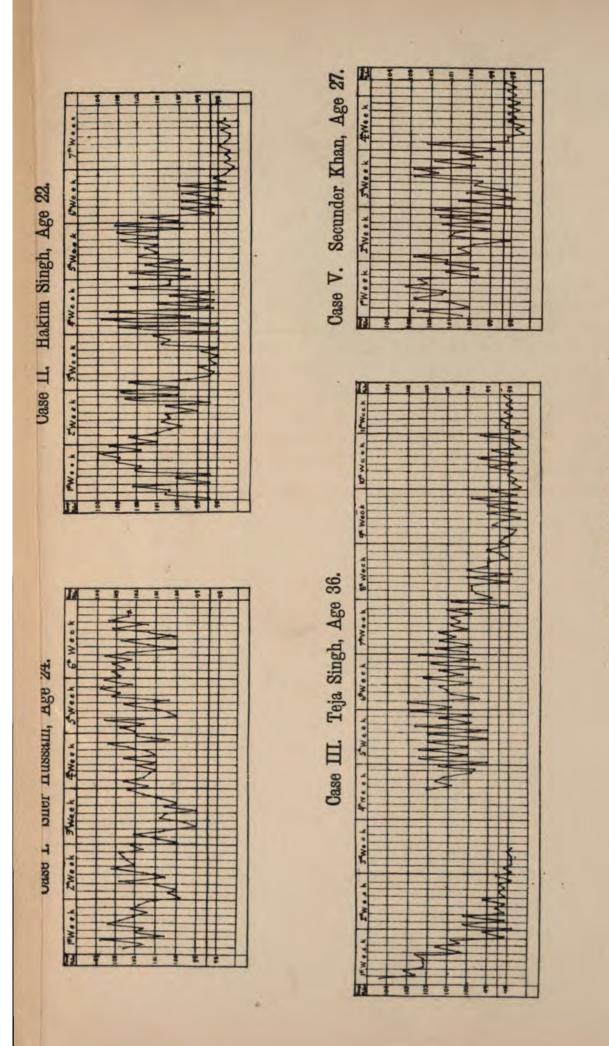
In conclusion, it is our pleasant duty to have to thank the following officers, without whose assistance the present investigation would have been impossible:—Major Adie, I.M.S., Captains Corry, I.M.S. Kerans, I.M.S. and Trafford, I.M.S., and Lieutenant McGillivray, I.M.S.

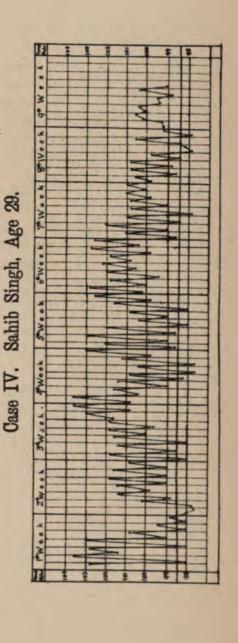
Note.—Since this paper was written nineteen other cases of continued fever have been investigated by us. The sera of all these patients gave a good reaction with M. Melitensis. They occurred in the following stations: in Ferozepore five cases in the 14th and 15th Sikhs; in Rawalpindi eleven cases in the 31st Punjabis; in Mian Mir one case in a British soldier; in Jullundur one case in a native follower; in Delhi one case in the 28th Punjabis. M. Melitensis was isolated from the spleen of the Delhi case. It is evident, therefore, that Mediterranean fever is both common and widespread, at any rate in the Punjab.

The part which goats play in the epidemiology of the disease had not been suggested when this paper was sent to press.

Notes and References.

- (1) The Lancet, March 6th 1897, p. 656.
- (2) British Medical Journal, April 10th, 1897.
- (3) The Lancet, September 9th, 1899.
- (4) Indian Medical Gazette, September 1900, p. 337.
- (5) Indian Medical Gazette, September 1900, p. 367.
- (6) Indian Medical Gazette, March 1901.
- (7) Zeitschrift für Hygiene; Vol. XLVI, 1904, p. 261.
- (8) Loc. Cit.
- (9) Comptes Rendus de la Société de Biologie, T. 59, 1905, p. 240.
- (10) Indian Medical Gazette, June 1904, p. 236.
- (11) Reports of the Commission for the investigation of Mediterranean fever under the supervision of an Advisory Committee of the Royal Society. Parts I & II, London, Harrison & Sons, 1905.
 - (12) British Medical Journal, February 5th, 1897.
 - (13) Loc. Cit.
 - (14) British Medical Journal, September 20th, 1902, p. 861.
- (15) Three cases of Mediterranean fever have occurred in the 15th Sikhs since this paper was sent to press. These occurred in September 1905, that is, five months after the regiment arrived in Ferozepore.
- (16) Reports of the Commission for the investigation of Mediterranean fever, Part I, p. 14.

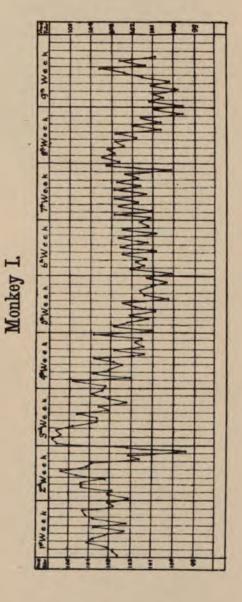


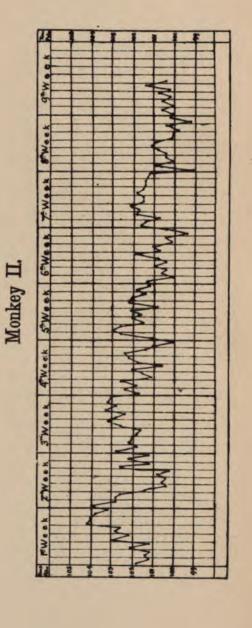


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Age 28. Mrs. P., Case XVI.

Amalak Shah, Age 17. Case XVII.

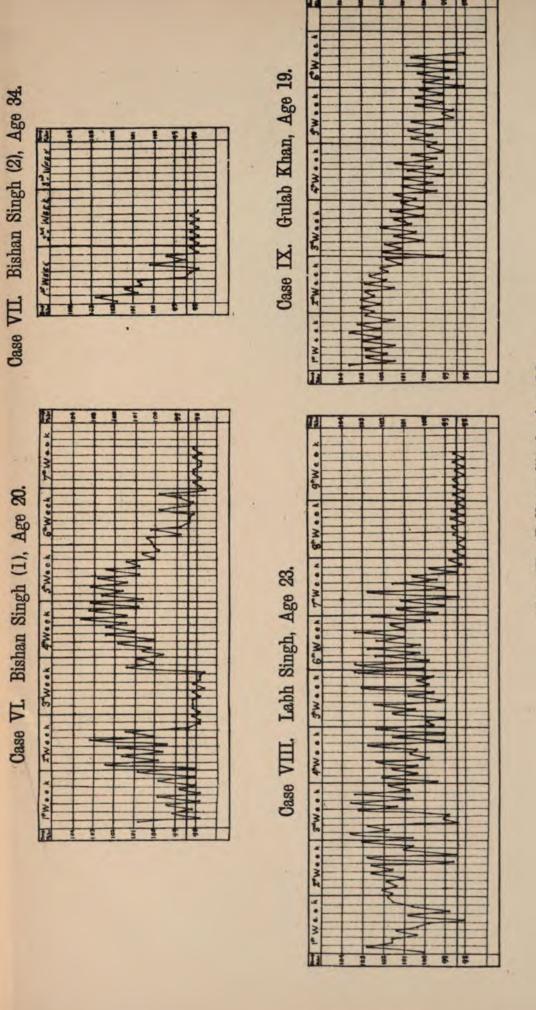




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Amalak Shah, Age 17. Case XVII. Monkey II. Monkey I. Case XVI. Mrs. P., Age 28.

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Case X. Dalhara Singh, Age 22.



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CAPTAIN GEORGE LAMB, M.D., I.M.S.
AND

ASSISTANT SURGEON M. KESAVA PAI, M.B., C.M. (Madras).

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